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# Male courtship vibrations delay predatory behaviour in female spiders

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During courtship, individuals transfer information about identity, mating status and quality. However, male web-building spiders face a significant problem: how to begin courting female spiders without being mistaken for prey? Male *Argiope* spiders generate distinctive courtship vibrations (shudders) when entering a female's web. We tested whether courtship shudders delay female predatory behaviour, even when live prey is present in the web. We presented a live cricket to females during playbacks of shudder vibrations, or white noise, and compared female responses to a control in which we presented a live cricket with no playback vibrations. Females were much slower to respond to crickets during playback of shudder vibrations. Shudder vibrations also delayed female predatory behaviour in a related spider species, showing that these vibrations do not simply function for species identity. These results suggest that male web-building spiders employ a phylogenetically conserved vibratory signal to ameliorate the risk of pre-copulatory cannibalism.

The information relayed between males and females during courtship may include species identity, mating status and quality<sup>1–3</sup>. The early and efficient transfer of such information during courtship is particularly important for predatory species. Predatory females are often highly aggressive toward males who risk injury, or in extreme circumstances death, during courtship<sup>4,5</sup>. This risk is most pronounced for male web-building spiders that have to enter the female's predatory trap, the web, to commence courtship. Female web-building spiders have excellent vibratory sensitivity but poor vision, and hence cannot visually distinguish a potential mate from prey<sup>6,7</sup>. As a result, males run a danger of being mistaken for prey upon entering the female's web<sup>8</sup>. Courtship has been hypothesized to ameliorate the risk of pre-copulatory attack from females, but the signals underlying this have remained elusive<sup>9,10</sup>.

Adult male orb-web spiders (*Argiope keyserlingi*; Araneidae) perform courtship shudders within seconds of coming into contact with adult female silk<sup>11</sup>. Males shudder by quickly rocking (anterior-posterior) in the web several times, a movement that generates a distinct vibration in the female's web, a form of 'tremulation' signal<sup>11,12</sup>. Males shudder often and sporadically during courtship<sup>11</sup>. In particular, courtship is characterized by a concentrated bout of shuddering during the earliest and riskiest phase of courtship when the male moves across the web to make his first contact with the female at the central hub<sup>11</sup>. Females prefer males that shudder at high rates, with consistent shudder durations<sup>11</sup>. We have previously identified a correlative relationship between the quality of shudders and female aggression: poor shudder performance during courtship is correlated with an increased risk of post-copulatory cannibalism<sup>11</sup>. These characteristics of shudders collectively suggest that shudders influence female aggression.

If this hypothesis is correct, we predicted that male shudder vibrations would influence female aggressive behaviour even toward prey in the web. To test this, we compared female latency to respond to live prey (a cricket) struggling in the web during simultaneous vibratory playback of (a) courtship shudders, and (b) white noise, compared to (c) a silent playback control. White noise was selected as a control vibration as it would provide a novel, point-source stimulus that spiders would not be expected to approach or attack. If shudder vibrations influence female aggressive behaviour, we expected to find that playback of shudders would delay female predatory responses to the cricket struggling in the web.

We further tested whether male shudders primarily function for species identification or as a generic signal that ameliorates female aggression. As all web-building male spiders face a similar identification problem when entering a female's web, we predicted that male web-building spiders generate generic, phylogenetically conserved vibratory signals to delay female predatory behaviour during the risky initial stages of courtship. To examine whether male shudder vibrations are species-specific, we tested whether shudders from male *A. keyserlingi* also delay female predatory responses in the congener *A. aetherea*.



## Results

**Playback of vibrations.** We recorded the courtship shudders of five males in a web using laser vibrometry. To ensure that male courtship shudders would be accurately reproduced in our assays, we compared the vibrations from the original recording, and a recording from the electromagnetic shaker used to artificially generate the stimuli in the experiment. The vibrations matched well between the two sources (Fig. 1; Supplementary Fig. 1).

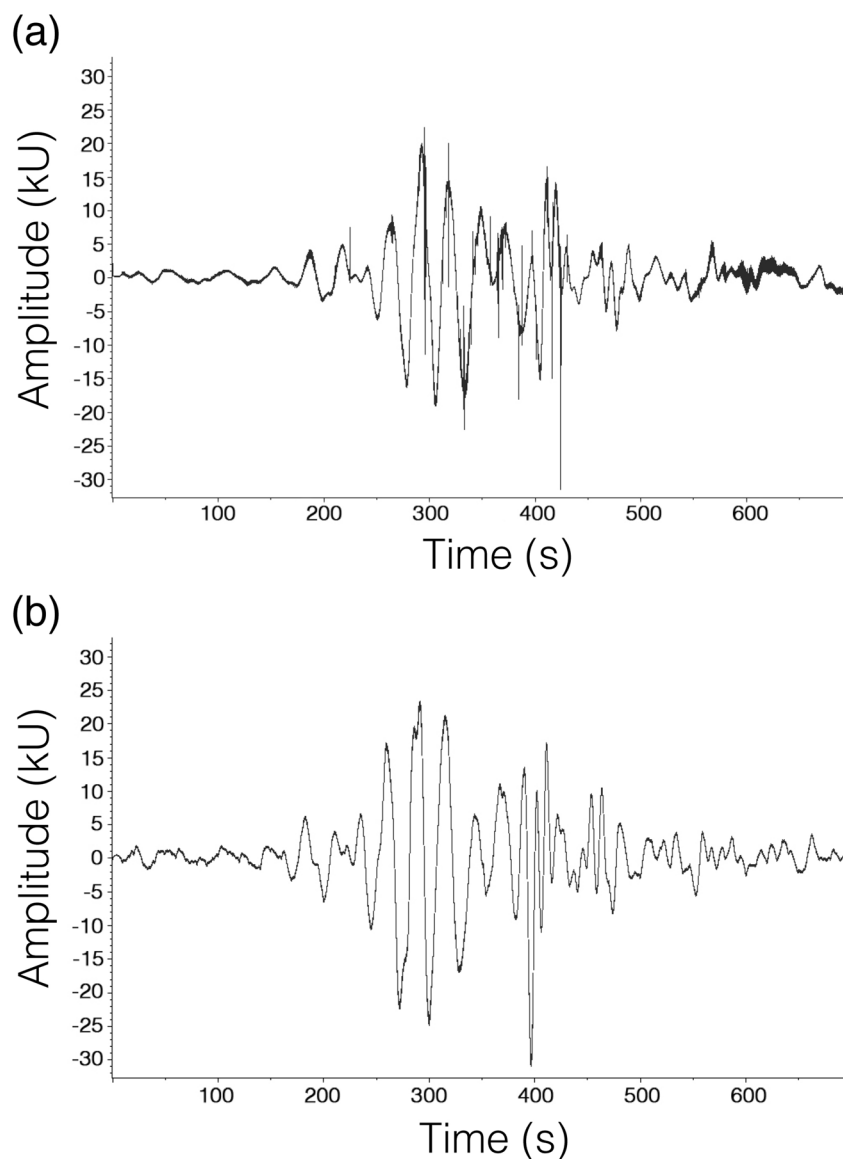
**Female *A. keyserlingi* predatory behaviour.** We found that females took significantly longer to respond when prey was presented simultaneously with male shudder vibrations compared to the silent playback control (Whole model:  $\chi^2 = 19.04$ ,  $df = 2$ ,  $p < 0.01$ ; Shudder:  $Z = 3.74$ ,  $p < 0.01$ ; Fig. 2a) but white noise did not affect female latency to respond to prey (White noise:  $Z = 0.99$ ,  $p = 0.32$ ; Fig. 2a). There was no difference between treatments in whether females attacked the cricket or not ( $\chi^2 = 2.44$ ,  $df = 2$ ,  $p = 0.30$ ).

**Female *A. aetherea* predatory behaviour.** We tested female *A. aetherea* predatory responses during playback of male *A. keyserlingi* shudder vibrations. As in our previous experiment, we found that

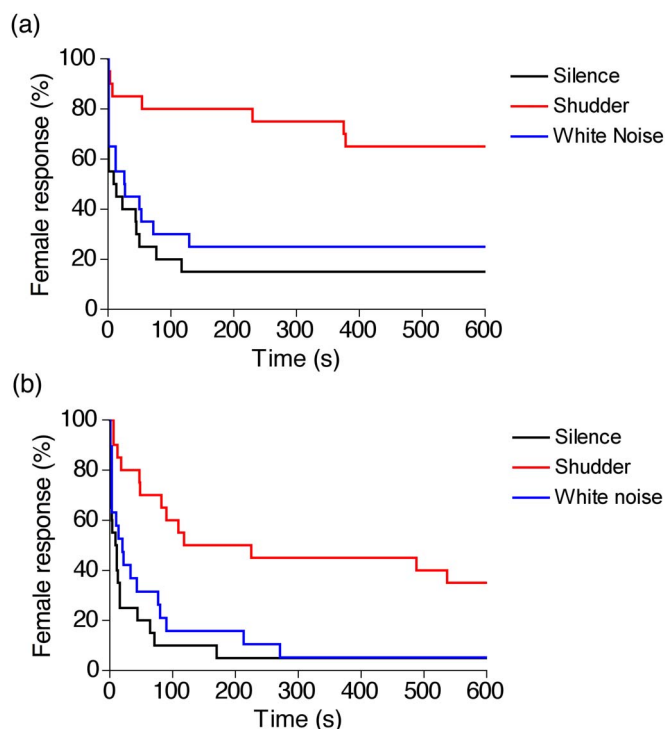
male *A. keyserlingi* shudder vibrations significantly increased the latency of *A. aetherea* females to respond to cricket prey compared to the silent playback control (Whole model:  $\chi^2 = 17.19$ ,  $df = 2$ ,  $p < 0.01$ ; Shudders:  $Z = 4.25$ ,  $p < 0.01$ ; Fig. 2b). Female responses to cricket prey when presented with white noise did not differ from silent playback control trials (White noise:  $Z = 1.56$ ,  $p = 0.12$ ; Fig. 2b). There was again no difference between treatments in whether females attacked the cricket or not ( $\chi^2 = 0.25$ ,  $df = 2$ ,  $p = 0.88$ ).

## Discussion

Our study shows that male web-building spiders generate a distinct vibratory signal that delays female aggression and thereby reduces the risk of female attack during the earliest and potentially riskiest stages of courtship. It is noteworthy that shudder vibrations delayed female predatory attacks, but did not definitively inhibit attack. There are three possible explanations for this. The first, and most probable, explanation is that the presence of male shudder vibrations alone is not sufficient to indefinitely suppress female aggressive behaviour. Our experimental design omitted many of the cues males provide during courtship (e.g., pheromones<sup>13</sup>, tactile stimuli<sup>14</sup>).



**Figure 1** | Comparison of natural and experimental stimulus waveforms. (a) Waveform of a vibration generated by an adult male shuddering in a female's web, and (b) waveform of the same shudder in (a) played through an electromagnetic shaker.



**Figure 2 | Female response latency.** Survival analysis showing the percentage of females that responded to the cricket prey over the test period. Each step indicates a female(s) responding to the cricket prey. (a) *A. keyserlingi* females responding to cricket prey during playback of *A. keyserlingi* shudders and white noise compared to the silent playback control, and (b) female *A. aetherea* responding to cricket prey during playback of *A. keyserlingi* shudders and white noise compared to the silent playback control.

Still, the observed delay in female predatory responses to shudder vibrations may be of biological relevance. In delaying female predatory behaviour, males have sufficient time to cross the web to the central hub where the female resides. Once at the hub, he is able to provide additional signals and cues that inform the female about his presence, identity and quality. This hypothesis is supported by the earlier finding that males shudder at a higher rate prior to reaching the hub, compared to after reaching the hub<sup>11</sup>.

A second possible explanation for the observed delay in female predatory responses is that the presence of male shudder vibrations may have inhibited the ability of females to detect and locate the prey struggling in the web (a ‘smokescreen’ effect<sup>15–17</sup>). However, this seems improbable, as the presence of white noise vibrations did not similarly delay female predatory responses. Further, our experiment presented shudders at 5 s intervals, thereby providing females with several seconds of prey-generated vibrations between shudder playbacks.

A third potential interpretation of our results is that the delay in female predatory behaviour during shudder playbacks is due to a confusion or disturbance (anti-predator) response. Some species of spiders, when disturbed, have been observed to freeze in response to a threatening stimulus<sup>18–20</sup>. Under this scenario, we would also predict delays in female predatory behaviour during white noise playbacks. However, shudder playbacks were the only treatment in which female predatory behaviour was delayed. Hence, we attribute the observed delays as a response to information contained within the shudder vibrations.

Our data on the generic nature of shudder vibrations studied in these two *Argiope* species suggest that there are constraints that limit the diversification of male courtship signaling. Ancestral spiders

were also predatory and utilized silk for prey capture<sup>21,22</sup>, hence it is likely that courtship signals to delay female aggression are basal for this group. A broad survey of courtship signals across diverse families of web-building spiders would help to elucidate this. The arguments for a phylogenetically conserved signal that delays female aggression are further strengthened by the requirement that male vibratory signals be distinct from prey vibrations. Due to their poor vision, vibratory stimuli are one of the few sensory channels by which females can determine the source of the stimulus in their webs<sup>6,7</sup>. Prey vibrations in orb-webs are characterized by an immediate impact vibration<sup>23</sup> and fast transients<sup>24</sup>, while those generated by courting males are characterized by temporal repeatability<sup>11,23</sup>. Signaling constraints (including, for example, morphology and receiver receptivity) are known to prevent signal diversification<sup>25</sup>. The reliance of females on vibrations to mediate predatory behaviour in webs may similarly limit the diversification of male courtship vibrations.

White noise, from the female perspective, may similarly be argued to be distinct from prey vibrations in structure due to its flat power spectrum. However, white noise produced no discernable effect on female predatory behaviour. This in turn suggests that there is specific information contained within the structure of male courtship shudders that is not contained within the structure of white noise.

A courtship signal that delays female predatory behaviours is adaptive from the male perspective in reducing the risk of pre-copulatory cannibalism. Similarly, delayed aggression in response to male shudder vibrations is adaptive from the female perspective. Female spiders that are highly aggressive toward prey-like stimuli in their webs may have a strong fecundity benefit<sup>4,26,27</sup>. However, the selective benefit of aggressiveness in predatory females may be offset by the cost of mistakenly attacking potential mates. Under this scenario, male shudder vibrations and the associated delay in female aggressive behaviours are mutually adaptive and may have co-evolved early in the evolutionary history of spiders.

Our study has identified a generic vibratory signal that significantly delays female aggression. The evidence collected here suggests that courtship shudder vibrations are highly conserved, a result that is somewhat surprising given that courtship signals are often expected to be species specific and a major trigger of reproductive isolation between species<sup>28–31</sup>. Our results also suggest that some of the first information the male communicates to the female during courtship is about appropriate predatory behaviour rather than individual identification. It has recently been suggested that the common assumption that mate identification occurs first during courtship should not be implicit<sup>32</sup>. Orb-web spiders hence offer exciting new opportunities to investigate the evolution, design and sequence of sexual signals.

## Methods

**Study animals.** We collected 20 female and 5 male *A. keyserlingi* (Sydney, New South Wales, Australia), and 20 female *A. aetherea* (Hervey Bay, Queensland). Spiders were fed once a week on crickets, houseflies or vinegar flies, and watered daily. The laboratory was maintained at 25–27°C and 50–60% humidity on a 12:12 h light:dark cycle.

**Stimuli.** Each female was tested three times in a repeated measures design (two vibration playback treatment trials - male shudder and white noise; and a silent playback control, with order randomised). To generate the vibration playback stimuli, we recorded the courtship vibrations of five male *A. keyserlingi* using a digital laser vibrometer (Polytec PDV 100, Germany). One shudder was randomly isolated from each recording. Recordings were digitized to hard drive through a Digital Rapids DC 1500 A to D board using Stream 1.5.23 (Digital Rapids, Canada) at 44.1 kHz/16 bits on a Windows computer (Dual 3.0 GHz Xeon, 4 GB RAM). We recorded transverse vibrations from the male courtship shudders, and playback was similarly mostly via transverse vibrations. Orb-web spiders appear to obtain much information from longitudinal vibrations, being more directional and faster at eliciting predatory responses<sup>7,33,34</sup>. However, insects trapped in webs generate mostly transverse vibrations<sup>7</sup>. In the present study, transverse vibrations were recorded due to the technological limitations of measuring longitudinal vibrations with large silk displacements from courting males<sup>7,11</sup>.



Each of the five shudder vibrations was presented as a playback stimulus to four randomly assigned females in both of the experiments. This ensured that we could represent natural variation in male courtship performance, but also avoid potential confounds from accidentally presenting a particularly high or low quality male shudder to females.

White noise was selected as a vibration control as it is a novel, point-source stimulus (i.e., not occurring across the entire web, such as wind or rain) that web-building spiders would not be expected to have an adaptive response to. A response to white noise would indicate that there is no specific information content within male shudder vibrations, and that temporal pattern alone is sufficient to alter female behaviour. White noise was generated using CoolEdit 2000 (Syntrillium Software Corporation, USA) and Soundbooth 2.0.1 (Adobe Systems Inc, USA). Five exemplars of white noise were generated, with the duration of each exemplar matched to the duration of a male's shudder. Both the shudder and white noise playback stimuli were normalized at  $-3\%$  in Soundbooth 2.0.1.

Stimuli were played back into webs using an electromagnetic shaker (V2, Gearing and Watson, UK) driven by an amplifier (30E, Gearing and Watson, UK) and mounted on an X-Y-Z manipulator that allowed accurate placement of the tip of the shaker on a silk thread. The shaker was controlled by a G4 Apple Powerbook computer. This playback method generates mostly transverse vibrations in the spider web. Stimuli were placed into five second sound files on a loop, so that every 5 seconds, the vibratory stimulus was repeated, simulating the mean shudder rate of natural male courtship<sup>11</sup>.

**Experimental procedure.** To set up a trial, we placed the tip of the electromagnetic shaker on a randomly selected radial thread of a female's web 15 cm from the hub and waited 10 minutes. This ensured that females had time to settle after the vibrations generated by placing the shaker tip on the silk thread. In treatment trials (shudder and white noise playbacks), we ensured one vibration was played prior to placing a live, struggling cricket prey in the web, and thereafter once every five seconds while the cricket continued struggling. The source of vibration from the electromagnetic shaker and the cricket prey came from two close points in the web (the cricket was placed within 1 cm of the shaker tip on the hub-side) thus reducing the possibility of females discerning multiple locations and hence sources of vibrations in the web that would make any differences in response difficult to interpret. During silent playback controls, we placed the tip of the electromagnetic shaker on the female's web to control for web contact. We then placed a live, struggling cricket on the web.

We recorded the latency for females to respond to the prey (move, orient and/or attack), or waited for 10 minutes. Timing began as soon as the cricket touched the web. In trials where the female attacked the cricket, we removed the cricket before the female began feeding. Each female received crickets of a similar size across the three randomised treatments. Females were rested for at least one hour between trials.

**Statistical analyses.** The data for female latency to respond to the cricket prey were non-normal and right censored. Hence, we analysed latency data with a survival regression, using a weibull distribution and included a frailty term for individual. Trials lasted 10 mins, after which any trials in which the crickets had not been attacked were aborted. Survival regression assesses the probability that a cricket will survive up to a particular time point (10 mins). The model tested the null hypothesis that there was no difference in the latency for females to respond to prey between the treatments (shudders, white noise) and silent playback control. Post-hoc analyses assessed which treatments showed a significant difference in latency to respond to prey compared to the silent playback control. Analyses were performed in R version 2.12.1 (The R Foundation for Statistical Computing 2010). All tests were two-tailed (alpha = 0.05).

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## Author contributions

A.E.W. and M.E.H. conceived and designed the experiments, discussed the results and wrote the manuscript. A.E.W. collected and analysed the data.

## Additional information

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